THE MORTALITY OF SEPSIS IN A PORTAL HYPERTENSIVE RAT MODEL

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1986

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A Thesis

Submitted to the Yale University School of Medicine
In Partial Fulfillment of the Requirement for
the Degree of Doctor in Medicine
April 1986
Department of General Surgery

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank these people who were intimately involved with the completion of this project. Without their help and guidance, this work may never have realized its fruition. I would like to thank Dr. Collin Smikle for his suport and diligence in motivating me, Dr. Richard Gusberg, for his help, resourcefulness, patience, and meticulousness in overseeing and correcting the final work, Dr. V.T. Marchesi for his time, effort, and advice in helping me to organize the project, the staff in clinical chemistry, hematology, and special thanks to Mrs. Ruth Adams in animal microbiology for her diligence and meticulous work. With the help of these people my thesis is finally complete.

ABSTRACT

Portal hypertension has been recognized as a clinical entity since the early decades of the twentieth century. Attempts to classify it based upon the location of the disease processes within the portal venous system resulted in prehepatic, intrahepatic, and posthepatic lesions being described.

The "forward" and the "backward" flow theories were proposed to explain chronic portal hypertension. The forward flow theory is based upon the development of a hyperdynamic splanchnic circulation while the backward flow theory is based upon the development of collaterals from portal systemic shunting, attempting to lower the portal pressure.

Based on the work done by previous authors, a prehepatic lesion was experimentally created by patrially ligating the portal vein of 44 Sprague-Dawley rats. 49 rats were used as controls. Portal hypertension was induced and then sepsis was induced by ligating and puncturing the cecum of 24 of the portal hypertensive and 24 of the control rats. Leucocytosis, percentage of immature bands, blood chemistries, blood and peritoneal cultures were derived from all the study groups at 12 and 24 hours.

Results demonstrated that there was a statistically significant difference in the degree of leucocytosis and the percentage of immature band forms between the control group and all the other study groups ($p < 0.05$) at 12 hours

as well as between the portal vein stenosed group of rats and the two septic groups. No difference was noted when the information from the blood chemistries were analyzed. Similar organisms were isolated from the blood and the peritoneal fluids from both groups with cecal ligation and puncture.

At 48 hours, only 1 rat from the cecal ligation and puncture group survived. There was no statistical significance between the control and the other remaining study groups.

Results from this study demonstrated that a prehepatic lesion resulting in portal hypertension did not significantly alter the immunodynamics of the host. Further studies are needed to explain the improved outcome in the portal vein stenosed group with cecal ligation and puncture over the other septic group.

Introduction

Portal hypertension has been recognized as a clinical entity since the early decades of the twentieth century.(1) In 1928 and 1932, McIndoe and McMichael respectively, used the term portal hypertension in their studies on the portal circulation.(2,3) The first manometric measurements in the portal circulation were reported in 1937 by Thompson et. al.(4) In 1944, the method of hepatic vein catherization to obtain blood samples directly from the hepatic vein in man was developed by Warren and Brannon.(5) Subsequently, Bradley reported on an indirect method of measuring hepatic blood flow.(6) By 1945, Blakemore and Whipple performed the first shunt surgery in a patient with portal hypertension.(7) Later that year, Whipple divided portal hypertension into two groups - intrahepatic and extrahepatic.(8) In the early 1950's many investigators utilized hepatic vein wedge pressures as a reflection of portal pressure in post - sinusoidal portal hypertension.(9,10,11,12) Since then, though much has been learned about the pathogenesis of portal hypertension, much is still unknown about portal hyptension in man, its complications and treatment.

Cirrhosis of the liver is the most common cause of portal hypertension in the United States and Western Europe.(13,14) Sixty to ninety five percent of the patients

with cirrhosis have a history of alcohol abuse.(13) The second most common cause of portal hypertension is mechanical obstruction of the extrahepatic portal vein. This obstruction may be a direct result of tumor invasion or thrombosis of the portal vein.(14) However, only five percent of the patients with cirrhosis and portal hypertension have portal vein thrombosis. The major causes of portal hypertension outside of the western hemisphere are schistosomiasis and Hepatitis B associated chronic active hepatitis.(13) To understand the pathogenesis of portal hypertension and cirrhosis, one must first be familiar with the vascular anatomy of the liver.

The portal vein is formed by the union of the superior mesenteric vein, which drains the intestinal tributaries, and the splenic vein. As the portal vein enters the liver it divides into many small branches which deliver blood into the hepatic sinusoidal system.(15) The hepatic artery, which has its origin from the celiac axis, enters the liver adjacent to the portal vein. The hepatic artery is also responsible for perfusing the hepatic sinusoids. After the blood has flowed through the hepatic sinusoids, the blood recollects by way of the hepatic venules, then through the hepatic veins and finally to the inferior vena cava en route to the heart.

The liver receives about 1500 ml of blood each minute, of which 2/3 is supplied by the portal vein. The hepatic artery supplies 40 - 60% of the oxygen supply to the

liver.(16) The portal vein differs from a systemic vein in three ways. First, the pressure in the portal vein is usually higher than systemic veins. Second, the oxygen content in the portal vein is normally higher than systemic veins. Finally, the portal veins are valveless. Due to the absence of valves in the portal system, an increased resistance to flow at any point between the splanchnic venules and the heart will increase the pressure in all vessels on the intestinal side of the obstruction.(16)

Portal hypertension represents a sustained increase in the hydrostatic pressure within the portal vein and/or its tributaries.(16) The increase in hydrostatic pressure is usually the result of an anatomic or functional obstruction to blood flow in the portal system at any point from its origin in the splanchnic bed to its exit into the systemic circulation.(15) Quantitatively, in humans, portal hypertension is considered present when the portal pressure is 5mm Hg greater than the pressure in the inferior vena cava. One can now localize the site of abnormal resistance to blood flow; and the portal hypertension can be classified according to the site of obstruction.(17,18) Prehepatic portal hypertension occurs when there is a functional or anatomic obstruction in the portal flow before it enters the liver e.g (thrombosis of the splenic or portal vein secondary to omphalitis, pyelophebitis, pancreatitis, trauma, tumor or hypercoagulopathic states).(19) These examples may result in

total or regional portal hypertension with clinical signs of portal systemic collaterals, splenomegaly, and hypersplenism.(19) The development of ascites is rare. The patient's first symptom may be an esophagogastric variceal hemorrhage. From a quantitative viewpoint, the pressure proximal to the obstruction is increased; however, the hepatic venous pressure gradient is normal.

Based on the anatomy of the liver, intrahepatic portal hypertension can be subdivided into three groups: presinusoidal, sinusoidal, and postsinusoidal. Presinusoidal intrahepatic portal hypertension occurs when there is a major resistance to flow in the portal venules. Schistosomiasis is considered to be the prototypical example. The degree of portal hypertension is proportional to the severity of schistosomal infestation and the load of ova deposited in the portal venules.(19) However the resulting PHT is due to the periportal granulomatous reaction against these foreign bodies and not due to mechanical obstruction. Other examples include congenital hepatic fibrosis, myeloproliferative disorders and metastatic liver disease. As in prehepatic PHT, the initial signs include the development of portal systemic collaterals, amd splenomegaly, with the formation of ascites being rare. Quantitatively, the hepatic veous pressure gradient is normal.

Sinusoidal intrahepatic, portal hypertension occurs when the major resistance to blood flow is in the sinusoids.

Alcoholic cirrhosis is the prototypal example. The initial signs include the development of portal systemic collateral circulation; with, vascular and metabolic consequences superimposed on the underlying cirrhosis.(19) Unlike the above groups, ascites is common. Quantitatively, the hepatic venous presure gradient and the portal venous pressure gradient are elevated.(19)

Postsinusoidal, intrahepatic portal hypertension is the result of the major resistance to blood flow in the hepatic venules. A brief list of examples include thrombosis of the hepatic venule within the liver, venoocclusive disease, (e.g following the ingestion of senechio alkaloids), congenital hepatic venous webs, and metastatic tumor. This clinical picture is indistinqiushable from Budd-Chiari syndrome.(19) The first signs include the development of portal systemic collateral vessels and ascites. Also, like sinusoidal intrahepatic PHT this entity creates an elevation in both the hepatic venous pressure and the hepatic venous pressure gradients.

The third type of PHT is post hepatic obstruction, caused by the blockage of blood flow in the inferior vena cava, above the site of entry of the hepatic veins.(19) Examples include constrictive pericarditis and severe congestive heart failure. Intractable ascites is common. Quantitatively, there is an increase in the absolute portal venous pressure but the hepatic venous pressure and the portal pressure

gradient are not elevated.

To understand the development and maintenance of portal hypertension, the underlying pathophysiology must be clarified. As previously noted, many diverse conditions can result in portal pressure elevation. Basic fluid mechanics used to describe the principles of blood flow can be applied to the pathophysiology of portal hypertension. The formulae,the change in pressure is equal to flow multiplied by the resistence. Thus, the effective pressure gradient between the two ends of a vessel depends on the interrelationship between the flow within the vessel and the resistance that impedes that flow. (20)

The pathogenesis of portal hypertension can be described on the basis of changes in vascular resistance. Vascular resistance can be altered by both physiologic and pathologic factors. The physiologic factors include the opening of capillary beds related to changes in metabolism, passive dilation or contraction of vessels in response to pressure changes, and changes in the state of contraction of smooth muscle in vascular walls mediated by vasomotor nerves and humoral substances.(20) The factors involved in pathologic changes are due to thrombosis of vessels, extravascular compression or intrinsic obstruction to flow secondary to collagen deposition.

When there is increased resistance to portal blood flow,

there is an increased portal venous pressure. As a result, preexisting collaterals dilate forming portal systemic shunts. These shunts are attempting to decompress the portal venous system. However, by so doing, they are carrying a major portion of blood away from the portal into the systemic veins. While these naturally - occuring shunts partially counteract the increased resistance to portal blood flow, the portal hypertension persists.(21,22) This persistence of elevated portal pressures implies that there must be a factor maintaining the portal hypertension, even in the presence of portal systemic shunting.

Though the maintainance of portal hypertension has been attributed to splanchnic hemodynamics, this subject remains controversial. Two theories have been proposed to explain chronic portal hypertension: the " backward flow " and the " forward flow " theories. The " backward flow " theory: The development of collaterals form portal systemic shunts which lower portal pressure. As the portal pressure is lowered, the hepatic resistance increases to maintain portal hypertension. The end result yields congestion of the portal venous system and a hypodynamic splanchnic and systemic circulation.(23,24) Works published by Bradley et al, in 1952, and Moreno et al in 1967 support the" backward flow " theory. The " forward flow " theory supports the development of a hyperdynamic splanchnic circulation. As in the backward flow model, portal systemic shunting occurs to lower the elevated portal pressure. However, there is an increase

in total splanchnic blood flow, hepatic and collateral, which maintains the portal hypertension. (25) There are several pieces of information in support of the forward flow theory. In 1958, Murray et al, working with chronic liver disease, found a hyperdynamic systemic circulation in patients with cirrhosis.(26) Gitlin et al in 1970, supported the forward flow theory while observing splenic blood flow and resistance in patients with cirrhosis before and after portocaval anastomoses.(27) The forward flow theory was further supported by Cohn et al in 1972 who reported increased splenic blood flow in patients with cirrhosis and alcoholic hepatitis. (28) Also in 1972 Wotelanski et. al. noted a shortening of the mean transit times of labeled albumin in the splanchnic circulation in cirrhotic patients.(29) The Vorobiof group, has recently reported a hyperdynamic splanchnic circulation in a portal vein stenosis rat model. They observed an increase in splanchnic blood flow in the maintainence of chronic portal hypertension.(20)

The sequelae of chronic portal hypertension are often times associated with portal systemic shunting and the maintainence of elevated portal pressures. This is secondary to a decrease in blood supply to the liver from the portal vein. This decrease in hepatic blood flow may be associated with an increased hepatic arterial component in order to maintain the portal blood pressure at near normal levels.(15) As mentioned earlier, the splanchnic circulation has been theorized

to be a factor contributing to the maintanence of the elevated portal pressures. Another important contributing factor is portal shunting of by - products of intestinal origin around the liver and into the systemic circulation. The development of splenomegaly and ascites is not uncommon in the face of portal-systemic shunting.

Clinically, an important consequence of portal systemic shunting is the formation of collaterals at any site along the gastrointestinal tract.(19) " The collateral blood vessels usually develop between the coronary vein of the portal system and the azygos veins of the caval system in the submucosa of the lower esophagus and upper stomach." (15) The thin walled vessels are better known as esophagogastric varices. These varices are inadequately supported by connective tissue and are a common site for a lethal hemorrhage in portal hypertensive patients. Gastric and hemorrhoidal varices are also sites of hemorrhagic derangements. One third of deaths in patients with portal hypertension secondary to alcoholic cirrhosis are related to variceal hemorrhage.(14) However, the most important consequences of these collaterals may be functional derangements rather than hemorrhagic. These derangements include portal systemic encephalopathy, the hepatorenal syndrome, ascites, spontaneous bacterial peritonitis, and septicemia.(19,27)

Portal systemic encephalopathy is principally the result of portal systemic shunting of blood around the liver cells

into the systemic circulation, and the presence of hepatocellular dysfunction. (15) Hepatic coma is a common complication of hepatic encephalopathy. The coma accounts for fifty percent of the deaths in patients with cirrhosis.(14) The associated neurologic syndrome is due to the presence of one or more of the intestinal toxic products normally metabolized in the liver. Ammonia has been incriminated frequently in the pathogenesis of hepatic encephalopathy but is unlikely to be the sole etiologic factor as neurologic dysfunction has been noted with various levels of ammonia.(15) Attempts to isolate other predisposing factors have been disapointing.

The hepatorenal syndrome, or progressive renal failure with azotemia and oliguria, is often associated with hypotension and hyponatremia.(15) This is a frequent terminal event in patients with end stage liver disease. The overall prognosis during a hospitalization from liver failure or from a portal hypertensive complication is greater than ninety percent if the patient develops the hepatorenal syndrome.(16) The pathogenesis of this syndrome is unclear. At autopsy, the kidney has been found to be anatomically normal. Clinical studies have shown the renal function to improve as the hepatic function improves. There appears to be an intense intrarenal vasoconstriction and a redistribution of blood flow. Plasma levels of renin and aldosterone are elevated. This elevation may be secondary to a reduced effective plasma volume in some patients.(16)

In other patients, the effective plasma volume is normal and the causes for the renal vasoconstriction is unclear. (16) Ascites is decribed as an accumulation of serous fluid within the peritoneal cavity.(30) The formation of ascitic fluid is believed to be due to an increased hydrostatic pressure and a decreased intravascular osmotic pressure. Fluid retention occurs as a response to an unknown hepatic sinusoidal baroreceptor; thus, as the plasma volume increases the ascites overflows.(19) The consequences of ascites are many. There is an increased risk of all types of abdominal hernias, a rise in the absolute pressure, which may precipitate hemmorhage from varices, the hepatorenal syndrome, and spontaneous bacterial peritonitis (SBP).(19) SBP is thought to be present when there is bacterial contamination of the ascitic fluid. The mortality of ascites with spontaneous bacterial peritonitis is aproximately seventy five to ninety five percent during a hospitalization.(13) This fatal complication of ascites involves microbes of enteric origin primarily aerobes. The presence of portal-systemic shunting bypasses the hepatic reticuloendothelial system (RES). The absence of this RES filter is believed to predispose the individual to infection and the development of septicemia in patients with portal - systemic shunting.(15,19) Though sepsis is a well recognized fatal complication of portal hypertension in man. Little is known about the development of sepsis in portal hypertensive patients.

Sepsis is defined as the presence of various pus forming and other pathogenic organisms, or their toxins, in the blood or tissues.(30) Sepsis, like any other infectious process, is the result of an interaction between microbial challenge and host defense mechanisms.(15) However, septicemia refers to a systemic disease caused by the multiplication of microbes within the circulating blood.(30) Intraabdominal sepsis is an infection external to the lumen in the gastrointestinal tract and within the abdominal cavity.(31) Most of the abdominal infections are the result of normal colonizing flora. The pathogenesis of intraabdominal sepsis involving the serosal surfaces is based upon a breech in the normal mucosal barrier secondary to an associated disease process. The microbial innoculum, chemical irritants, lymphatic drainage, and the inflammatory response are important pathogenetic factors in the development of intraabdominal sepsis.(31) The relative roles of hepatic dysfunction, portal -systemic shunting, and other splanchnic or systemic hemodynamic changes which predispose to sepsis remains poorly defined

Animals models in scientific research:

The use of animals models in experimental research has become an integral part of the study of disease processes in humans. Animal experimentation, although limited in clinical application by differences in species, has been responsible for much of our present knowledge of pathologic deviation from normal function.(32) Reproducible models for portal hypertension and intraabdominal sepsis have been avidly

sought. Several animals have been used to create a portal hypertensive model. The methods utilized can be divided into intrahepatic, extrahepatic and/or a combination of the two types.(33) The models for an intrahepatic portal hyper-tensive rat consists of injecting hepatotoxins or foreign matter into the portal vein or liver, and the use of deficiency diets.

In 1976, Koo and his group injected carbon tetrachloride into the liver to induce cirrhosis.(34) Recently, Shibayama has used deficiency diets in rats to localize increased hepatic resistance in cirrhosis.(35) He created cirrhosis by feeding the rats a choline deficient diet.

Extrahepatic models of portal hypertension usually involve mechanical manipulation of the portal, hepatic or splenic vein. These models are created by stenosis or ligation of a vessel. Many groups have used extrahepatic manipulation of the portal vessels. In 1976, Saku attempted to induce portal hypertension and esophageal varices by using ameroid constrictors around the portal trunk.(36) He reported complete constriction of the portal trunk with a twice normal increase in portal pressure. Angiography revealed splenorenal collaterals with collaterals overbridging the ameroid constrictors in all rats with the constrictors.

Orda and Ellis induced portal hypertension by partial constriction of the portal vein.(37) Angiographic studies demonstrated the spontaneous development of portal systemic and porto-pulmonary shunting. A coincident decompression of

the portal system was associated with the shunting. However release of the portal vein stricture led to the disappearance of the collaterals in the majority of the animals.

Halvorsen and Myking, in a series of experiments, developed a model of prehepatic portal hypertension by using a calibrated stenosis of the portal vein. A stenosis to 1.2mm lead to a sustained elevation in portal venous pressure two times the control. This increase in portal venous pressure lasted approximately eight weeks. Subsequent studies done by this group compared a graded stenosis in tubes to vessels of small calibres.(39) This was an in vitro vs. in vivo comparison of changes in flow variation. In vitro studies showed a prestenotic pressure variation with a coefficient of variation in repeated stenosis less than three percent. In animals stenosis repeatedly produced the same high level of portal pressure. As a result, Halvorsen and Myking concluded their portal vein stenosis model to be satisfactory when compared to the theoretical standard.(39)

A two stage portal vein ligation with subsequent total occlusion in the rat was described by Kibria in 1980.(40) This model of portal hypertension demonstrated a collateral circulation of varicose, anastomotic vessels. Marked esophageal varices developed in six out of twenty three animals. Uvelius et. al. ligated the hepatic branches of the portal vein to create a portal hypertensive model.(41)

By 1981, Hamilton created a partial ligation of the portal vein to induce portal hypertension.(42) He reported increased prostacyclin levels one week after ligation of the

portal vein. Hamilton postulated, if this process occured in man, then this may be responsible for local wound vasodilation and inhibition of platelet aggregation. This may be an important factor contributing to the severity of hemorrhage from esophageal varices.

In 1983, Vorobioff et. al. created a portal hypertensive model by stenosing the portal vein to the diameter of a twenty gauge, blunted, hypodermic needle.(43) Radioactive microspheres were used to determine splanchnic hemodynamics. They found generalized splanchnic arteriolar vasodilation occuring in the presence of high grade portal systemic shunting. Studies showed increases in portal venous inflow with elevated portal venous pressures were not due to changes in portal vascular resistance. These findings supported the forward flow theory for maintenance of chronic portal hypertension.

Sikuler's group partially ligated the portal vein to create a model of portal hypertension.(44) Radioactive microspheres were utilized in order to study portal hemodynamics. They found increased portal venous inflow to be responsible for the maintenance of chronic portal hypertension. These results support the forward flow theory for maintenance of chronic portal hypertension and, are in agreement with result reported by Vorobioff et. al.

Benoit studied forward and backward flow mechanisms of portal hypertension and the relative contributions in a portal vein stenosis model.(45) Portal venous inflow, portal

systemic shunting and portal venous pressure were all elevated ten days post stenosis of the portal vein when compared to controls. Portal venous resistance was forty percent higher in portal vein stenosis animals. Increased portal vein resistance was due to high resistance in the portal venous collaterals. Model predictions indicated the forward flow to account for forty percent of the increased portal pressure, and the backward flow to account for sixty percent of the increased portal pressure.

The selection of an animal for expeimental research is important. The rat can be considered as one of the most suitable animals for experimental research.(46) This is primarily because this animal is strong, inexpensive, easy to handle , breed requiring little room, and offers the possibility of assembling large series of simiiar animals.(46) Male rats are preferred because of their docility, stability of endocrinologic state, and a more pronounced growth rate.(46) Among the numerous strains of outbred rats, the Sprague - Dawley and Wistar strains are the most commonly used for experimental liver investigations.(46)

Septic animal models:

Dogs, baboons, pigs, rabbits, guinea pigs, and mice have been used to study sepsis. (47-60) However, we will limit our discussion to rat models. There are three well recognized and reproducible animal models for intraabdominal sepsis. These models were developed by Bartlett et. al.(61) Wichterman,s group (62) and Short et,.al (63)

Bartlett's group standardized intrabdominal abscess formation with generalized sepsis. Gelatine capsules containing B. fragilis and E. coli in a standard mixture with rat colonic content and barium sulfate, a known irritant added to increase the toxicity of the implant, were implanted intra-abdominally.(61) There is an initial acute peritonitis, E.coli bacteremia, and a high mortality. This model would enable one to study the roles of various microbes in terms of septic complications after colonic perforation.

Wichterman et al, reviewed the literature for reproducible and clinically relevant septic models. His group approved only the aforementioned model. However, the model proposed by Bartlett et al although satisfactory , was not a simple model.(62) Wichterman's group developed an animal septic model by using cecal ligation with subsequent puncture. The bacterial challenge following cecal ligation and puncture is continuous, and of such tremendous magnitude that this initiating trauma is almost always lethal.(62) This model is simple, inexpensive and reproducible. This is a good model if one wanted to study alterations in tissue metabolism, energy production, and hormonal responses during sepsis.(62) These studies are possible because this model enables one to study sepsis in an initial hyperdyanmic circulation and a later hypodynamic circulation. Work done by Wichterman was reproduced by Martinell's group in 1985.(64)

In 1983, Short et. al. standardized an intraperitoneal E.Coli injection model of septic shock.(63) This model is

suitable to study pharmacological treatment of septic shock.(63) Martinell's group also found this work to be reproducible.(64) Both models were found to closely mimic the clinical situation as found in posttrauma and postoperative periods.(64) The two attributes of both models include the gradual development of shock and the time allowed for the aminal to use natural defense mechanisms inorder to overcome the disease.(64) These models are thought to be useful in studying pathophysiologic mechanisms and in evaluating treatment regimens in posttraumatic and postoperative septic shock.(64)

The sepsis models mentioned above were all induced in healthy animals. In human conditions, sepsis usually develops in the face of an already present disease process. As mentioned earlier, little is known about intraabdominal sepsis in portal hypertension. This study will address the impact of sepsis on portal hypertensive rats as compared to controls.

As stated previously, portal vein stenosis has proved to be a reliable method of inducing portal hypertension. (38, 39, 42-45) Given the relative ease in creating this model as well as the reproducibility of the technique, it was decided to create a model for portal hypertension utilizing the technique described by Halvorsen et.al. (38) Benoit described an increase in the portal venous pressure and portacaval shunting after 10 days.(45) In accord with his observations, and to allow adaptation to the new hemodynamic state, sepsis was induced in the portal hypertensive models in 15 - 18 days.

The technique for ligating and puncturing the cecum, as described by Wichterman et.al., will be utilized to study the differences between the portal hypertensive and the control groups given the lethality, reproducibility, ease, and cost of this procedure. Given the relative short time course between the time that the cecum was punctured and the death of the animal, it was decided to study the animals at 12 and 48 hours as this would demonstrate the animals' initial response to the bacterial load and their subsequent response. It was postulated that, within 12 hours, the animal would react to the bacterial challenge by recruiting the circulating leukocytes to fight the foreign substance. After 48 hours, maximum recruitment from the initial circulating leukocytes should have occurred and the body would respond by generating new cells, increasing the percentage of immature bands seen in the peripheral smear.

MAATERIALS AND METHODS

Male albino Sprague-Dawley rats (Charles River Laboratories, Cambridge, MA) were housed in screened top cages and allowed free access to food (Purina Rat Chow, St.Louis, MO) and water throughout the of experiment. The surgical techniques described below were performed on rats weighing between 300 and 350 grams. After each portion of the experiment requiring manipulation of the rats, the animals were placed in clean cages. The animal facility was temperature and humidity controlled. The animals were divided into the following groups.

Group A: Portal vein stenosis

Group B: Cecal ligation and puncture

Group C: Portal vein stenosis and cecal ligation and puncture

Group D: Sham control

Anesthesia:

An airtight plastic container with a hinged lid, measuring 10 cm x 15 cm x 25 cm, was used as the ether chamber to initially anesthetized the rats prior to manipulation. Cotton padding, measuring approximately 1 cm in depth, was placed at the bottom of the container. Prior to manipulation, approximately 2 cc of ether was placed into the container. The rat was remved from the cage and placed into the ether filled chamber for 30 seconds. After this time, the animal was removed from the chamber and injected with 0.1cc/0.1kg body weight of ketamine hydrochloride (Ketaset)

intramuscularly. An additional 0.15 cc of Ketaset was given if additional anesthesia was required during surgical manipulation of the animal.

Production of portal hypertension:

Group A: 19 rats underwent partial portal vein ligation to induce portal hypertension and portal systemic shunting. This method of inducing portal hypertension has been described in detail by previous work done by Chojkier and Groszmann.(65) Each animal was anesthetized as described above. The abdominal cavity was opened through a two centimeter midline incision under sterile conditions. The omentum and part of the intestine were gently lifted out of the abdomen and kept moist with warm normal saline gauze pads. After separating the hepatic artery and the bile duct, the portal vein was exposed. A twenty gauge blunt tip hypodermic needle was placed alongside the length of the portal vein and one ligature of 3-0 silk was placed proximal to the bifurcation of the vein and secured around the needle and the portal vein. The needle was removed, and the portal vein was allowed to reexpand. The abdominal viscera were placed back into the abdomen. The abdomen was then closed in two layers with 3-0 silk. Once hemostasis was acheived, the animals were given cc/cc normal saline volume replacement for blood loss.

Cecal ligation and puncture:

Group B: In accord with the technique described by Wichterman et. al.(62), the cecum of 24 were isolated, ligated and

punctured. At operation, the rats were anesthetized and a two centimenter midline incision was made under sterile technique, and the cecum was divided carefully avoiding all blood vessels. The cecum was filled with feces by gently milking stool back from the ascending colon. The cecum was then ligated just below the ileocecal valve with a 3-0 silk ligature. Ligation at this point permitted bowel continuity to be maintained. The antimesenteric cecal surface was punctured once with a 25 gauge hypodermic needle. A small amount of fecal content was expressed from the cecum and the bowel was replaced into the peritoneal cavity. The abdomen was closed in two layers with 3-0 silk. All operated rats received 5cc of normal saline/ 100 gram body weight subcutaneously plus an additional cc/cc normal saline replacement for blood loss.

Portal Vein Stenosis plus Cecal Ligation and Puncture:

Group C: The above portal vein stenosis technique was performed on 24 rats. 15 - 18 days after the portal vein was stenosed, cecum was ligated and punctured using the technique described above. These rats received 5ccNS/100grams body weight subcutaneously plus cc/cc normal saline volume replacement for blood loss.

Sham control rats:

Group D: The protocol for isolating the portal vein was performed on 25 rats. However, the portal vein was isolated but not stenosed. The abdomen was closed in two layers with 3-0 silk. These rats were given cc/cc NS volume replacement

subcutaneously for blood loss. At 15 -18 days, these rats were reoperated with the CLP technique. The cecum was isolated, milked full on stool in the usual fashion, and divided carefully avoiding all blood vessels. According to the protocol described by Wicterman et. al., the cecum was replaced into the abdominal cavity.(63) The abdomen was closed in two layers with 3-0 silk. All rats were given 5ccNS/100grams body weight subcutaneously plus cc/cc NS volume replacement for blood loss.

Study protocol:

93 rats were included in the study. These rats were distributed into the various groups as noted in Table 1. In accord with the divisions described in Table 1, 30 rats were sacrificed at 12 hours. The surviving rats from the 24 hour group were sacrificed at 24 hours.

The 29 rats remaining in the mortality study were followed for 1 week. These rats were observed daily and the number alive at the end of each day was recorded. After 1 week, the mortality rate was calculated by dividing the number of rats remaining in each group by the number of rat within the groups in the beginning of the 1 week period.

Blood Samples:

At the schedued time for sacrificing, each rat was lightly anesthetized in the ether chamber. Cardiac puncture was then performed and the blood withdrawn was divided into smaller aliquots aseptically and sent to the various

laboratories for analysis.

Hematology:

Aliquots of blood were sent to the Hematology Lab for analysis. The number of leukocytes was determined spectrophotometrically. However, the differential for the types of white blood cells were counted manually and recorded as the percentage of the total number of leukocytes.

Chemistry:

Samples of blood were also sent to the Chemistry Lab where sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3), lactic dehydrogenase (LDH), alkaline phosphatase (Alk Phos), blood urea nitrogen (BUN), creatinine (Cr), serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) were determined using an autoanalyzer.

Blood cultures:

Aliquots of blood from each study animal were sent to the Animal Microbiology Lab for anaerobic and aerobic culturing. Anaerobic cultures were placed into the Columbia single vacutainer unit (American Scientific Products) with cysteine added. Blood samples for aerobic culturing were collected in the Bac-tek blood culture system (Whevetron Co.). The cultures were examined daily and subcultures were done 2 days and 7 days after culturing. In addition, subcultures were done whenever there was evidence of growth in the culture bottles (turbidity or gas production). Samples were placed on McConkey's, blood agar, colimycin-naladixic acid (CNA)

agar plates. All cultures were incubated at 37 degrees centigrade. Microbial identification was done using standard diagnostic methods.

Peritoneal Cultures:

Peritoneal specimen were obtained on Culturette swabs (American Scientific Products) and innoculated unto blood agar, McConkey agar, CNA, and Kanamycin agar plates for recovery of aerobes. For culturing anaerobes, a Kanamycin plate was inoculated and the swab placed in thioglycol broth. The plates for anaerobic growth were placed in Gas-Pack jars (BBL Laboratories, Cockeysville, Md.). All cultures were incubated at 37 degrees. Bacterial identification was done using standard diagnostic techniques.

Statisical analysis

All the results are expressed as mean and standard deviation. A two-tailed Student T test was used to analyze differences between each group. Statisical significance is described as a P = 0.05 corresponding to a 95% confidence level.

RESULTS

Leukocytosis at 12 and 48 hrs:

Tables 2 and 5 report the leukocyte counts of each experimental group at 12 and 48 hours respectively. As noted in Table 1, the mean leukocyte count in Group D was 15.16 $\pm$ 2.75 wbc/mm . This was significantly different from the mean leukocyte counts in Groups A - C which were 10.67 $\pm$ 2.57 wbc/mm, 2.8 $\pm$ 1.67 wbc/mm, and 5.83 $\pm$ 3.2 wbc/mm respectively. There was also a difference noted between Group A and both Groups B and C ($p < 0.05$) at 12 hours.

Table 3 illustrates the disparity in the number of immature bands in the peripheral smear of the various groups at 12 hours. As shown, greater than 15% of the total number of circulating leukocytes were immature bands in 80 % of the rats in both Groups B and C while the number of immature forms was less than 5% in Groups A and D. As a result, there was a statistically significant difference in the amount of bands between the combined groups [Groups A & D vs Groups B & C] ($p < 0.05$).

Only 1 rat from Group B was alive at the end of 48 hours. As a result, no comparison could be made between this group in the other groups. In comparing the leukocyte counts between the 3 other groups at 48 hours, no statistically significant difference was noted.

Electrolytes and Liver Function Tests:

The electrolytes and liver enzymes were measured from blood samples drawn at the time of sacrificing. As illustrated in Table 9, no statistical significant difference was noted between the groups.

Bacteremia at 12 and 48 hours:

Tables 4 and 6 illustrate the incidence of bacteremia in each group at 12 and 48 hours. As noted, Groups A, B, and C had a higher incidence of bacteremia than did Group D. The most frequently cultured organism at 12 hours was Escherichia Coli , with Bacteriodes species and Streptococci species being the other common organisms cultured. Proteus mirabilis and Clostridia were also demonstrated. No organisms were cultured from the blood of the control organisms. As reported in Table 4, 50% of the rats in Group A were bacteremic compared to 100% of Groups B and C. Only 1 out of 8 rats from Group D had evidence of bacteremia. Staph. aureus was cultured from that rat.

At 48 hours, blood cultures were available from only 3 rats from both Groups B and C. No growth was noted from the only surviving rat in Group B. No growth was noted in the blood cultures from Group D and a single organism was isolated from the blood of each of the 3 bacteremic rats in Group A.

Peritoneal Cultures:

Tables 10 and 11 illustrate the results of the

peritoneal cultures at 12 and 48 hours. As noted in Table 10, 100 % of the rats from Groups B and C had positive peritoneal cultures at 12 hours. Enteric organism and bowel flora were the most commonly isolated organisms. Only 14.3 % of the rats in Group D and 33.3 % of the rats from Group A had positive peritoneal cultures and Staphylococcus aureus grew from one of the two positive cultures in each group.

Table 11 illustrates the results of the peritoneal cultures from the study groups at 48 hours. Only 1 rat from Group B was alive at the end of 48 hours and Enteric organisms were isolated from its peritoneum. All 3 rats from Group C had positive cultures. Growth was noted in the cultures of 2/5 rats from Group D and 1/4 rats from Group A.

Mortality Study

The mortality rate of each of the four groups were studied over a one week period. Each group was prepared as described in the Materials and Methods. The rats were placed in clean cages and the mortality rate was recorded. The results found are illustrated in Figure 2. This demonstrates that all the rats from Group B were dead after 3 days, with an overall mortality rate of 100% over the one week period. The rate of death in Group C was less with only 35% dying during a similar period. None of the rats from either the PVS or the sham groups died during this time course.

DISCUSSION

The disparity in the leukocyte counts noted between Groups B and C, compared to the control group, Group D, was as expected. Similar results were reported by Hansson et.al. and Martinell et.al. in their experimtents on abdominal sepsis.(66, 64) Though both of these authors inoculated their animals with pure cultures, they found that, after 12 hours, the degree of leukocytosis was less in the septic model than in the controls. The reduced number of circulating leukocytes is secondary to (a) margination into the areas of infecton, and (b) phagocytosis of intravascular microorganism by circulating polymorphonuclear cells. Work done by Postel et.al supports this explanation for the reduction in white cell count.

The increase in the number of immature cell forms are expected in the two septic groups, Groups B and C, as the body attempts to combat the infection by releasing immature granulocytes (> 15% bands). This difference was noted in the above groups as compared to Groups A and D at the 12 hour interval (< 5% bands). As the bacterial load in the latter groups is much lower, the difference in the number of immature white blood cells is as expected.

In their report on peritonitis using the CLP technique, Wichterman, et.al. stated that the predominant organisms cultured from the blood and peritonuem of the septic rats were E. coli, Strep. bovis. P. mirabilis, and B. fragilis

.(62) Tables 4, 6, 10, and 11 illustrates that similiar findings were noted in this study group with E.coli and B. fragilis being the predominant organisms isolated. Though they inoculated pure cultures into the peritoneal cavity, similiar hemodynamic results were described from their experiments, suggesting that a similiar pathophysiologic process was taking place.

Though all the rats in the study groups were alive after 12 hours, few rats survived from the septic group at 48 hours. Therefore it was difficult to evaluate leukocytosis, the number of immature bands, or any other parameters in Group B at this time period. No significant difference was noted between Group C, the other septic group, and the other groups. This was difficult to explain as both Group B and Group C underwent similar techniques to induce sepsis. We had sought to demonstrate differences between the portal hypertensive groups compared to the controls. The hypothesis stated that portal hypertension would result in decreased clearing of the organisms, resulting in an increased bacterial load during the late phase of sepsis (> 48 hours). As a consequence, the survival of this group would be less than or equal to the control group (Group C < Group B). The reverse was noted in most of the parameters evaluated. Explantions fof this departure from the expected outcome include (1) choice of techniques for inducing sepsis. Though utilized by Wichterrman et.al. in their studies, repeated puncturing of the cecum may have introduced an innoculum size

that proved too overwhelming for the animal. However, this doesn't explain the disparity between Group B, rats that had undergone cecal ligation and puncture, and Group C, rats that had underwent stenosis of the portal vein prior to cecal ligation and puncture.

The second explanation pertains to the choice of techniques for inducing portal hypertension. Prehepatic stenosis of the portal vein reduces the flow of blood from the intestines and the the attached mesentery. Therefore, the amount of bacteria invading the systemic circulation may have been less, or the rate of release into the circulation may have been slower. This allowed these rats to mount an effective response by the cellular immune system as well as the reticuloendothelial system. Since similar organisms were cultured from the blood and the peritoneal fluid of both Groups B and C compared to the control groups (A and D), one can conclude that it was the size of the innoculum rather than the type of organism that was the crucial factor in the differences noted in survival [Tables 4,6,10 & 11]. Pathologic examination of the liver, spleen and abdominal viscera, as well as other organ systems would provide further insight into the discreptancies from the expected versus the observed outcome in between the two groups.

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Table 1. Number of rats in each study group.

Group	12 hrs.	48 hrs.	1 wk.	Total
Cntrl	7	12	6	25
PVS	7	6	7	20
CLP	8	8	8	24
PVS-CLP	8	8	8	24
Total	30	34	29	93

Table 2. Leukocytosis at 12 hrs.

Group	# of rats	Mean	Range	S.D.	p value vs. cntl
Cntl	7	15.16	7.6-18.9	2.75	-
PVS	7	10.67	7.9-17.1	2.57	0.05
CLP	8	2.8	0.8-4.4	1.67	0.001
PVS-CLP	8	5.83	2.1-8.9	3.2	0.001

Table 3. Presence of Bands at 12 hrs.

Groups	Mean % Bands	Range	Trend
Cntl	0.3	0-4	7/7 = < 5%
PVS	0.4	0-5	6/6 = < 5%
CLP	17.43	8-26	6/8 = > 15%
PVS-CLP	19.63	8-31	6/8 = > 15%

Table 4. Blood cultures at 12 hrs.

Organism	Cntl	PVS	CLP	PVS-CLP
No growth	7	2	0	0
E.coli	0	3	7	6
Clostridia	0	1	2	1
B.fragilis	0	1	4	3
Strep. spp.	0	1	3	3
Proteus mir.	0	0	2	1
Lactobacillus	0	1	0	1
Staph. aureus	1	0	1	1
% of rats bacteremia	12.5	50	100	100
Total # rats	8	4	6	8

Table 5. Leukocytosis at 48 hrs.

Group	# of rats	Mean	Range	SD	p value vs. cntl
Cntl	12	11.63	5.4-15.3	3.53	-
PVS	6	10.68	6.5-15.6	3.66	< 0.05
CLP	1	2.4	-	-	
PVS-CLP	6	12.12	7.8-19.2	5.82	< 0.05

Table 6. Blood cultures at 48 hrs.

Organism	Cntl	PVS	CLP	PVS-CLP
No growth	6	3	1	0
E.coli	0	0	0	1
Strep.spp.	0	0	0	1
Staph.aureus	0	1	0	1
Pseudomonas	0	1	0	0
B.fragilis	0	0	0	2
Enterobacter	0	1	0	0
Total	6	6	1	2

Table 7. Comparison of leukocytosis in various study groups at 12 hrs.

Investigator	Mean leukocytosis		p. value vs. control
Alexander	control	15.16 (± 2.75)	-
	PVS	10.67 (± 2.57)	< 0.05
	CLP	2.8 (± 1.67)	< 0.001
	CLP-PVS	5.83 (± 3.20)	< 0.001
Hansson et.al [66]	Septic	2.7 (± 1.2)	< 0.001
	Control	7.9 (± 2.5)	-

Table 8. Blood Chemistries at 12 hours.

	Group A	Group B	Group C	Group D
Sodium	139 (± 3.5)	141 (± 3.0)	140 (± 4.0)	140 (± 3.0)
Potassium	4.2 (± 0.3)	4.4 (±0.4)	4.3 (±0.4)	4.5 (± 0.5)
Chloride	108 (±10.5)	106 (± 8.6)	115 (±11.2)	110 (±5.8)
Bicarb-onate	24 (±2.8)	22 (±3.3)	21 (±2.6)	26 (±2.2)
SGOT	213 (±18.5)	382 (±36.1)	353 (±24.7)	240 (±53.6)
Alk. Phos	68.5 (±7.9)	61.3 (±8.2)	69.8 (±11.3)	70.6 (±13.0)
BUN	15 (±2.5)	20.3 (±5.4)	14.4 (±3.4)	15.6 (±2.9)
Creat.	0.6 (±0.17)	0.6 (±0.20)	0.7 (±0.29)	0.7 (±0.12)

Table 9. Blood Chemisrties at 48 hours.

	Group A	Group B	Group C	Group D
Sodium	137 (±4.5)	139	141 (±3.2)	138 (±4.0)
Potassium	4.0 (±2.1)	3.8	4.1 (±1.6)	4.0 (±2.3)
Chloride	110 (±6.0)	113	108 (±7.3)	111 (±4.1)
Bicar-bonate	20 (±3.7)	19	22 (±2.9)	23 (±5.1)
SGOT	234 (±34.5)	QNS	318 (±49.6)	256 (±27.6)
Alk. Phos	65 (±6.7)	QNS	64 (±8.1)	68 (±4.7)
BUN	17 (±2.1)	QNS	15 (+1.5)	14.1 (+0.9)
Creat.	0.5 (±0.11)	QNS	0.7 (±0.18)	0.6 (±0.14)

Table 10. Peritoneal Fluid Cultures at 12 hours.

	Group A	Group B	Group C	Group D
No Growth	4	0	0	6
Stap. aureus	1	4	6	1
Prot. mirabilis	0	4	3	0
Escherichia coli	1	8	5	1
Strep. faecalis	0	5	7	0
Clostridia spp.	0	1	0	0
Bact. fragilis	2	6	5	0
Total	6	8	8	7

Table 11. Peritoneal Fluid Cultures at 48 hours.

	Group A	Group B	Group C	Group D
No growth	4	0	0	3
Eschericia coli	0	1	2	0
Strep. faecalis	0	1	1	0
Bact. fragilis	0	1	2	1
Clostridia spp.	0	1	1	0
Enterobacter	1	0	1	0
Staph. aureus	1	1	0	2
Total	4	1	3	5

Fig. 1 Comparison of WBC at 12 and 48 hours.

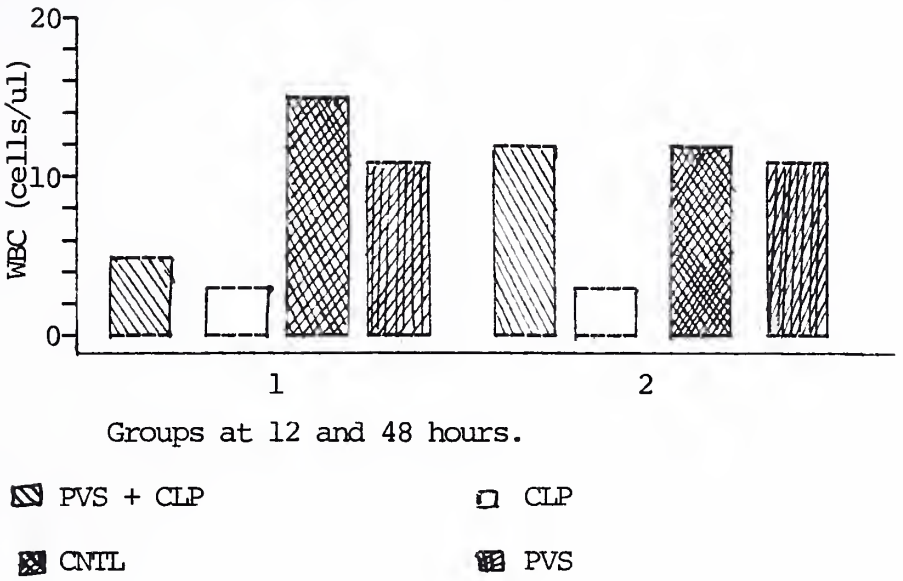
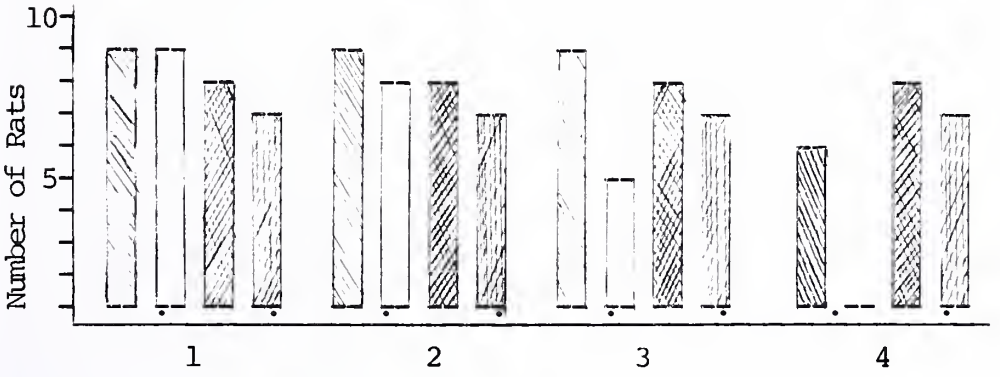


Fig. 2 Mortality of Groups at 1 week.



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